IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re Application of: CEVC, Gregor

Serial No.: 07/844,664

Filed: April 8, 1992

For: PREPARATION FOR THE APPLICATION OF AGENTS IN MINI DROPLETS

Examiner: G. Kishore Art Unit: 1615

DECLARATION UNDER 37 C.F.R. § 1.132

Hon. Commissioner of Patents
And Trademarks
Washington, D.C. 20231

October 21, 1999

S I R:

I, Gregor Cevc, Ph.D., declare as follows:

1. I am the named inventor of the above-referenced patent application, and I am the Chief Executive Officer of IDEA AG, Germany, assignee of the above-referenced patent applications.

2. I have read and understand the Office Action dated May 25, 1999 in which the Patent Examiner rejected claims of this application as being anticipated by and/or obvious in view of EP Patent No. 0 220 797, alone or in combination with additional references (Mayer (BBA, 1986)) with or without Patel (FEBS Letters). The Examiner noted that while it was "unclear whether EP teaches all of the instant functional parameters . . . in the absence of showing the criticality, they are deemed to be parameters manipulatable by an artisan to obtain the best possible results." I further understand that the Examiner has also relied on EP 0 220 797 and Mayer in combination further in view of U.S. Patent No. 4,911, 928 (Wallach). Wallach is relied upon as disclosing vesicles containing edge active substances (e.g., surfactants) such as

those disclosed in the above-referenced patent application, and further teaches that active agents such as pheromones, insulin and pesticides can be encapsulated in the vesicles.

3. Lipid vesicles, on the basis of phospholipids and/or detergents, are described in several patents. In Transfersomes™, which are disclosed and defined in my above-referenced patent application, the combination of these compounds is optimized with respect to membrane flexibility, which allows the vesicle to penetrate barriers (e.g. skin) more easily than a conventional formulation.

4. EP 0 220 797 describes a suspension containing a phospholipid and a hydrophilic nonionic surfactant. U.S. Patent No. 4,911, 928 (Wallach) describes a method for the production of paucilamellar lipid vesicles from non-phospholipid surfactants.

5. In order to demonstrate important differences between my invention (as claimed in the above-referenced patent application) and EP 0 220 797 and Wallach, I conducted investigations described below. Formulation A is substantially the same as Example 1 of EP 0 220 797, the sole difference being the substitution of hexane for chloroform (which material is removed from the final product). Formulation B is substantially the same as Example 1 of U.S. Patent No. 4,911, 928 (however, in order to be more accurate in manufacturing, the volumes were doubled).

6. Materials

6.1 Formulation A[1] (according to EP 0 220 797)

• Soybean lecithin from Nattermann	8.00 g
• Cholesterol from Fluka	2.00 g
• Tocopherol from Synopharrm	1.00 g
• Soybean oil from Synopharm	1.00 g
• Polyoxyethylene 8 Lauryl-ether from Sigma	1.00 g
• Chloroform from Merck	51.68 g
• Injectable water from Delta-Pharma	100.g

[1]Lab.-ref.: 0019-21

6.2 Formulation B[2]: paucilamellar vesicles (according to U.S. 4,911,928 (Wallach))

• Polyoxyethylene (2) cetyl ether from Sigma	1.39g
• Cholesterol from Fluka	0.146g
• Dicetyl phosphate from Sigma	0.110g
• 5 mM phosphate, 150 mM NaCCl, pH 7.4	10 mL

6.3 Formulation C[3]: Transfersomes batch 44-01 (according to my above-referenced patent application, U.S.S.N. 07/844,664)

• Triamcinolone acetonide from Synopharm	200 mg
• Soybean phosphatidylcholine (SPC) from Natterman	18.87 mg
• Tween 80 from Synopharm	31.13 g
• Ethanol from BfB	18.87 g
• Benzylalcohol from Merck KgaA	2.63 g
• 50 mM Phosphate buffer, pH 6.5	428.30 g

7. Methods

7.1 Particle Size

Vesicles size was determined by the dynamic light scattering (DLS) according to QCM-3005 with equipment from ALV-laser GmbH (ALV-500 Multiple Tau Correlator; 10 mW He-Ne laser; detection at an angle of 90°). The calibration was done with latex particles of defined size (Polysciences Inc.). The temperature of the goniometer cell was adjusted to 23 °C with a circulating-water bath, the sampling time was 20 seconds.

For each trial, approx. 20 μL of vesicle suspension was diluted in 1 mL of bidistilled water and measured immediately. The data was analyzed by two different mathematical procedures which were Cumulant and ILT analysis. Mean particle sizes presented in this report were taken from the cumulant analysis of 1th order.

7.2 Product description

A few milliliters of the suspension were filled in a glass beaker and examined visually.

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[2]Lab.-ref.: 0014-48/54

[3]OC document

7.3 Penetration assay

The penetration capability of lipid vesicles was tested with a miniaturized filtration device, according to QCM-3201. In short, the suspension was driven by a known pressure through an Anopore membrane with 20 nm pores, smaller than the average vesicle diameter.

8. Preparation of lipid vesicles

8.1 Formulation A

The suspension was prepared by dissolving the organic compounds listed in paragraph 6.1 in chloroform. The solution was dried by rotavaporation and under a stream of nitrogen. After storage in a dessicator overnight, a light yellow, slightly oily mass was obtained. 5 g of this mass was mixed with 100 g water to get a milky, slightly yellow suspension. 50 g of the suspension was treated 3 times with an Ultra-Turrax homogenizer (from Janke and Kunkel GmbH, IKA-Laborotechnik) using different speed and time, as given in the following table. The particle size was measured after each treatment.

treatment	rpm	time(sec)	particle size (nm)
1	8000	60	834
2	11200	60	504
3	14400	30	294

8.2 Formulation B

The polyoxyethylene (2) cetyl ether, cholesterol and dicetyl phosphate were mixed together at 40° C. 0.824 g of this mixture was injected into 10 mL of 5 nM phosphate buffer containing 150 mM NaCl, pH 7.4. The phosphate buffer, which was contained in a 10 mL syringe, was also at 40° C. The resulting mixture was forced into a second 10 mL syringe through an orifice about 1 mm in diameter. The suspension was driven continuously back and forth between the two 10 mL syringes for approximately 3 minutes. A milky and viscous suspension resulted. Upon sonication for 4 minutes in a continuous cycle at 60 watt with an

IKA-Labortechnik sonicator from Janke and Junkel GmbH, the lipid vesicles were reduced enough in size to pass through a 0.2 μm filter.

8.3 Formulation C

The suspension was prepared by mixing together triamcinolone acetonide, soybean phosphatidycholine, Tween 80 and ethanol (see paragraph 2.3) until a clear solution was obtained. Then, benzylalcohol and buffer were added to the solution and the resulting suspension was mixed overnight. The suspension was extruded through a 01 μm filter.

9.0 Results

Results of particle size measurements, penetration assay, and product description are summarized in Table 1.

Table 1: Results of the physico-chemical characterization of various tested lipid vesicles

	Formulation A: Liposome suspension	Formulation B: Paucilamellar vesicles[4]	Formulation C: Transfersomes[5]
mean particle size	294 nm	280 nm	167 nm
penetration assay	0.02 mg/sec*MPa*cm^2 at 1 MPa	does not pass through the filter at 1 MPa	170 mg/sec*MPa*cm^2 at 0.4 MPa
production description	very slightly yellow, opalescent homogenous suspension	white, milky, homogenous suspension	Translucent, white to pale yellow liquid

Conclusion

10. The Transfersome suspension (C) was white, slightly yellow and opalescent, which is typical for formulations with the given composition. The liposome suspension (A) was

[4]Lab.-ref.: 0012-21 to 23 and 0016-25

[5]Lab.-ref.: 0014-48 to 54

similar to this, whereas the paucilamellar vesicles (B) were much more turbid and non-opalescent.

11. The mean particle size of the lipid vesicles in formulation (A) and (B) was higher than that of Transfersomes. Transfersomes particle size is within the range of 120 to 220 nm.

12. In the penetration assay, which measures membrane flexibility, the first suspension (A) passed through the filter only at the highest pressure applied in this assay (1 Mpa). The paucilamellar vesicles did not penetrate through the filter at 1 MPa. In contrast to the aforesaid liposomes, the formulation C revealed the typical behavior of Transfersomes: above certain threshold pressure value, the penetration capability is high and similar to that of pure water or of mixed lipid micelles with diameter much smaller than that of Transfersomes. Changing the size of the latter does not have a very major effect on the performance of the penetration assay, however.

13. Figure 1 shows the results of the penetration assay performed on liposomes (A) and Transfersomes (C). The paucilamellar vesicles (B) did not penetrate under these conditions.

14. The results show that the two formulations described in the literature clearly differ from Transfersomes with respect to particle size and penetration capability.

15. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

October 21, 1999
Date

Gregor Cevc, Ph.D.

Figure 1

Results of the penetration assay performed on liposomes (A) and Transfersomes (C). The paucilamellar vesicles (B) did not penetrate under these conditions.

Pressure (MPa)	liposomes: formulation A	Transfersomes (C): batch 44-01	water
0,04		25,4	
0,06		43,7	
0,15		122,2	540
0,2		152,5	540
0,4		170	540
0,6			
0,8			
1	0,02